
# Biomolecular Breadboards for Prototyping and Debugging Synthetic Biocircuits

University of Minnesota

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**Outline**

I. Project goals, objectives and applications
II. Task 1.1: Cell-free circuits breadboard (Murray)
III. Task 1.3: Artificial cells (Noireaux)
IV. Task 1.4: Biochemical wires (Rothemund)
V. Project risks and needs

# Project Goals and Objectives

**Develop, demonstrate, document, and disseminate two new "biomolecular breadboards" that provide engineers with 10-100X improvement in time required to conceive, design and implement working biomolecular circuits**

| Program metric | Current | Phase I | Phase II |
|---|---|---|---|
| 1. Time required to go from synthesized DNA sequences to measurement of circuit performance (on cell-free [and origami] breadboards) | 1-2 wk | 3 days | 1 day |
| 2. Time required to build a novel, modest complexity (6-8 unique promoter) circuit - existing design, novel components | 3-6 mo | 1 mo | 1 wk |
| 3. Number of circuits that can be tested simultaneously, varying component concentration and/or cell-free toolkit parameters | 5 | 25 | 100 |
| 4. Number of genes and regulatory parts characterized, modeled and available for use in cell-free circuits (and artificial cells) | 2 | 5 | 20 |

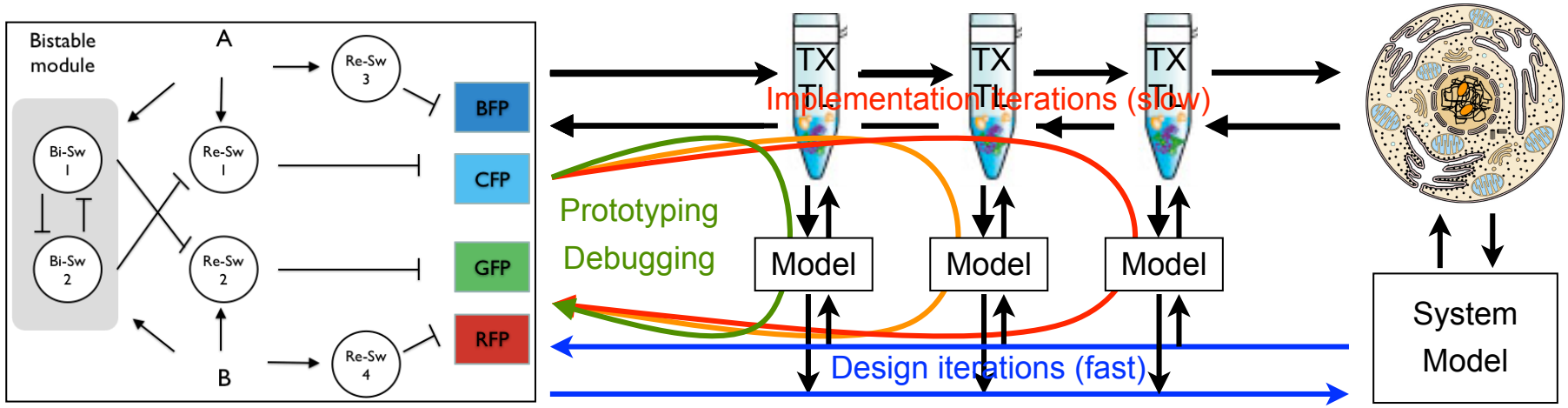
**Primary approach: Cell-free breadboard for modeling, prototyping and debugging**

- Build on *in vitro* TX-TL system ("cell-free toolkit") developed by Noireaux (UMN)
- Provide working components, composable models, and prototyping protocols for building low and moderate complexity circuits and transitioning to cells

**Add'l technologies: DNA origami breadboard (II), artificial cells, biochemical wires**

- Allow (TX-TL) prototyping with spatial effects: femtoliter volumes + spatial localization

# Task 1.1: Cell-Free Circuits Breadboard




**Key characteristics of the cell-free breadboard (Noireaux et al)**

- Inexpensive and fast: ~$0.03/ul for reactions; typical reactions run for 4-6 hours
- Easy to use: works with many plasmids or linear DNA (PCR products!)
  - Can adjust concentration to explore copy number/expression strength quickly
- Flexible environment: adjust energy level, pH, temperature, degradation

**Milestones/demo for Phase I** http://www.openwetware.org/wiki/breadboards

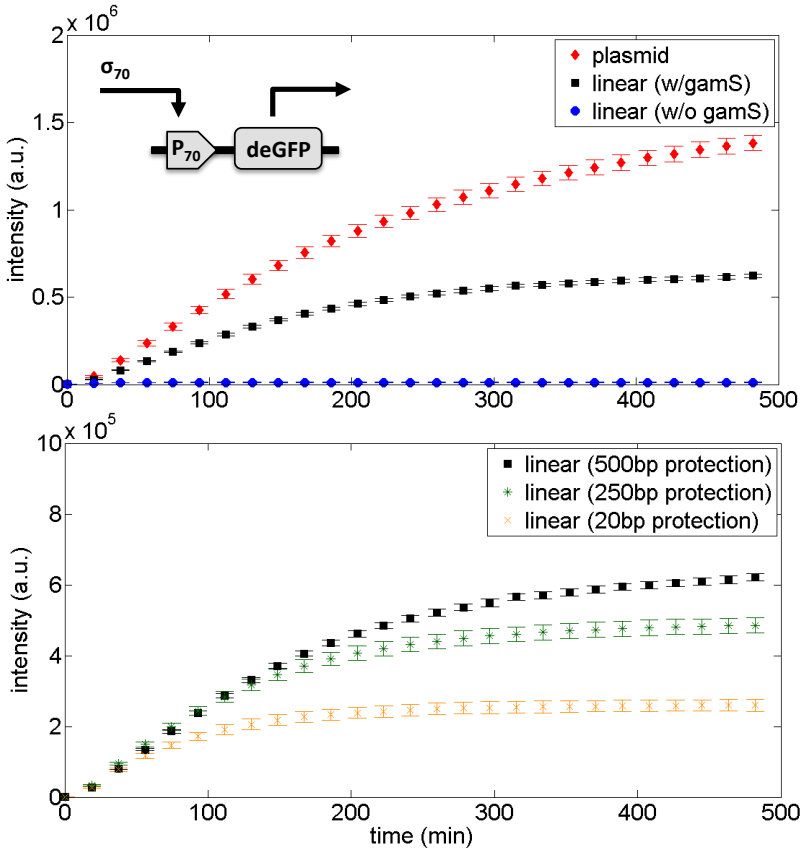
- Q1: Post protocol on web, along with controls + summary of costs
- Q2: Demonstrate breadboard on 2 circuits (eg, switch, IFFL), document iteration time
- Q3: Post complete protocols + variations (degradation, energy, ...) + validated models
- Q4: Demonstrate design of 6-8 promoter circuit with 3 day cycle time, 1 month total

**Phase II demo: 8-16 promoter circuit, 100 variations, 1 day cycle time, 1 week total**

# Preliminary Results: Cell-Free Breadboard

## Cell-free technology development

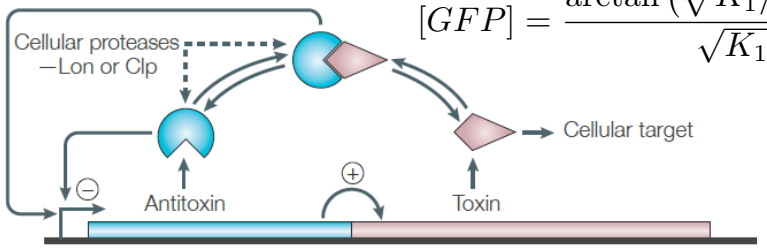
- Use linear DNA for rapid prototyping
- Problem: exonucleases degrade DNA
- Solutions: gamS blocks exonuclease activity or use protection sequences
- Ongoing: RNA/protein degradation



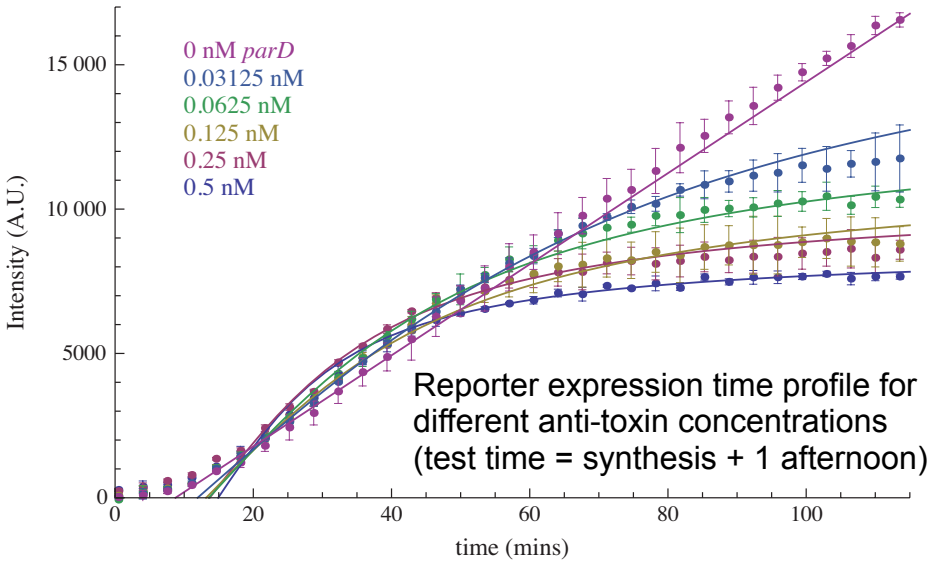

## Case study: toxin-antitoxin modeling

- Exploring dynamics of toxin-antitoxin system in *E. coli*; tricky to characterize
- Use TX-TL to characterize circuit and identify constants/transfer curves
- Anti-toxin auto-regulation:

$$[GFP] = \frac{\arctan\left(\sqrt{K_1/K_2}(t - t_d)\right)}{\sqrt{K_1 K_2}}$$




Gerdes et al., Nat Rev Microbiol (2005)




Reporter expression time profile for different anti-toxin concentrations (test time = synthesis + 1 afternoon)

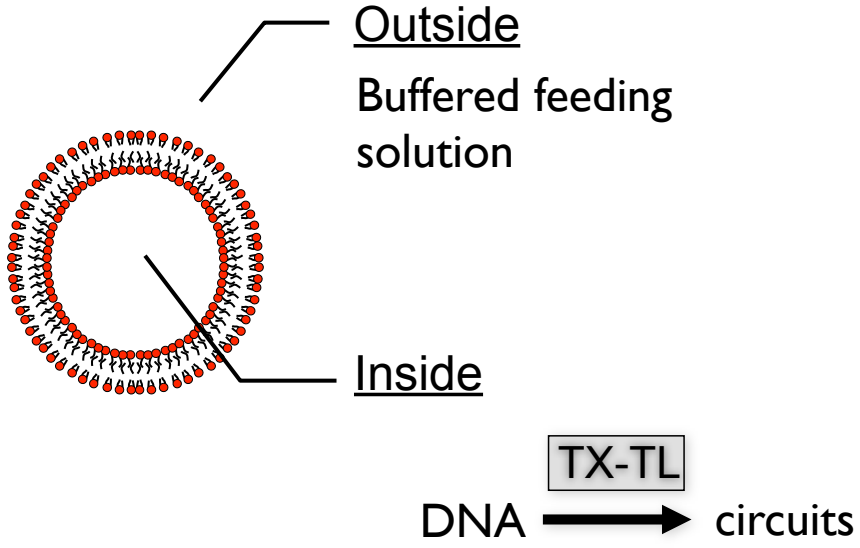
# Task 1.2: Artificial Cells

**Demonstrate the feasibility of a programmable synthetic phospholipid vesicle system with elementary synthetic gene circuits**

- Create cell-sized (1-50 μm diameter) synthetic phospholipid vesicles containing TX-TL system and genetically encoded circuits
- External inducers (tetracycline, arabinose, ...) will diffuse through the membrane and activate the circuits or repress expression of fluorescent protein reporters

**Approach**

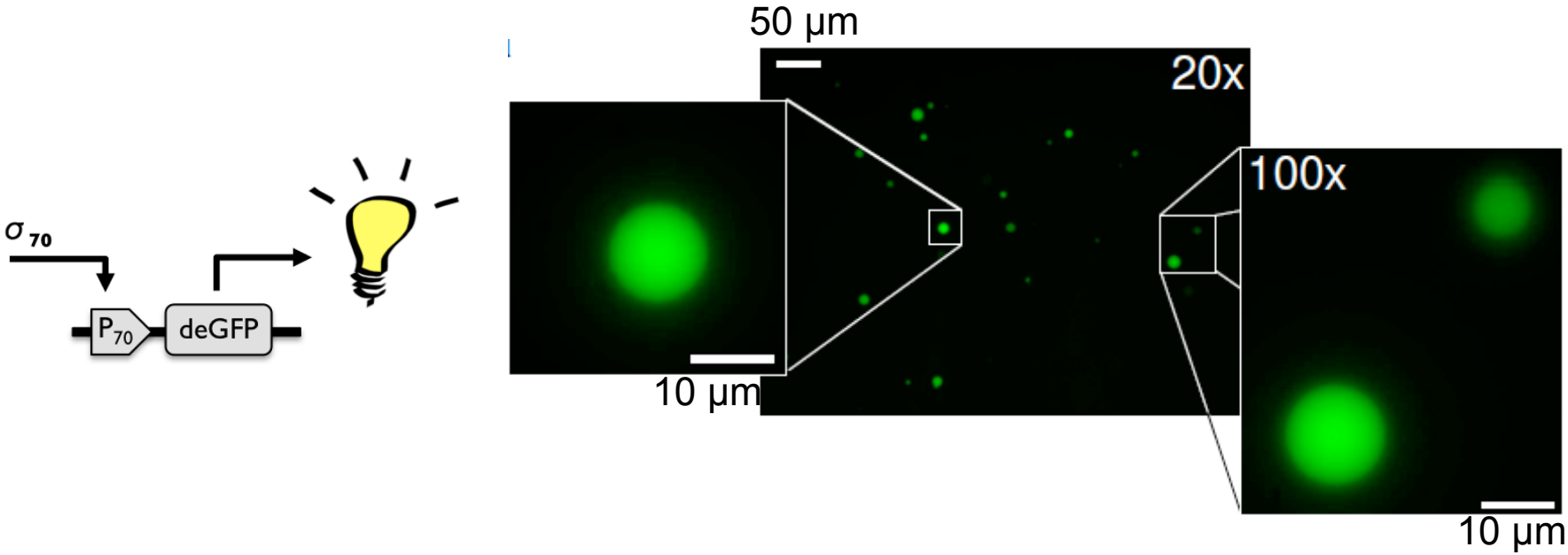
- Demonstrate stable synthetic liposomes capable of hosting transcriptional activation and repression units
- Demonstrate activation and repression units that can be turned on and off using inducers diffusing through the membrane (arabinose, lactose, tetracycline, …..)

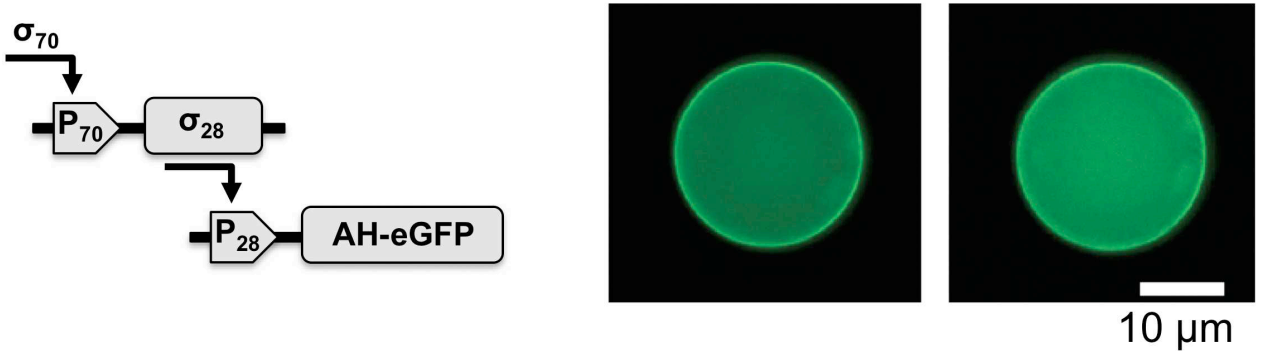



**Extensions**

- Changing the phospholipid composition. Now PC, objective: add 1 or 2 other lipids. The protocol of vesicle preparation will be adapted if needed
- Understand the importance of the phospholipid composition for pattern formation, self-organized systems at the membrane

# Artificial Cells: Initial Results



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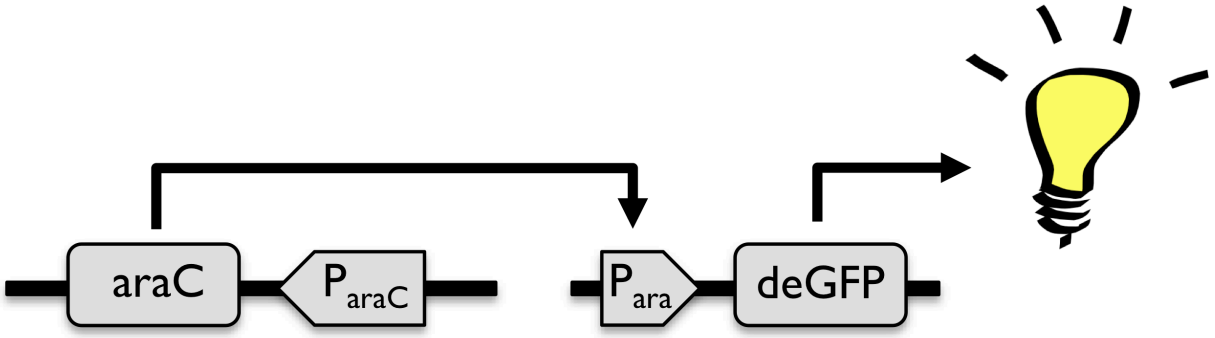


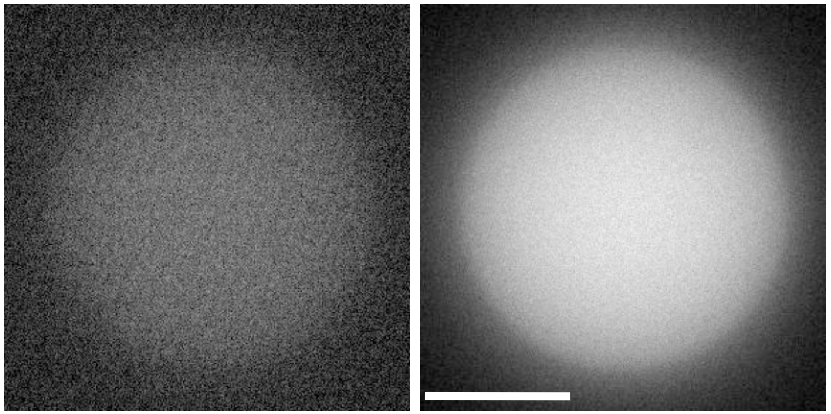


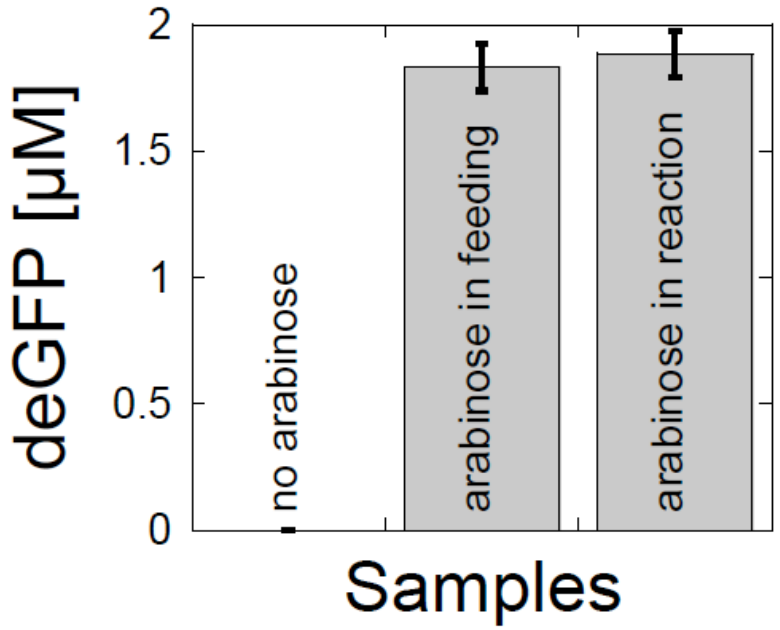

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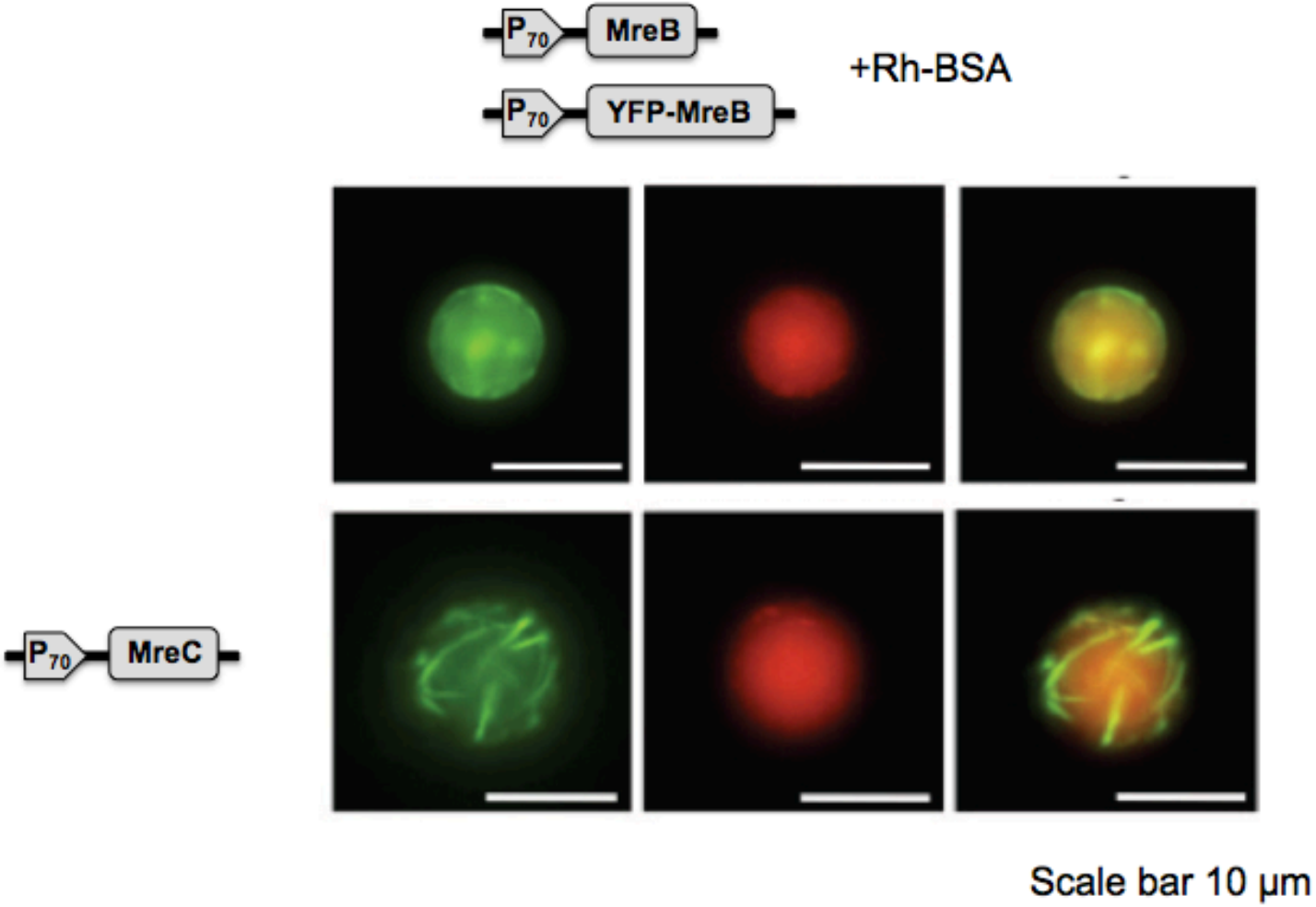
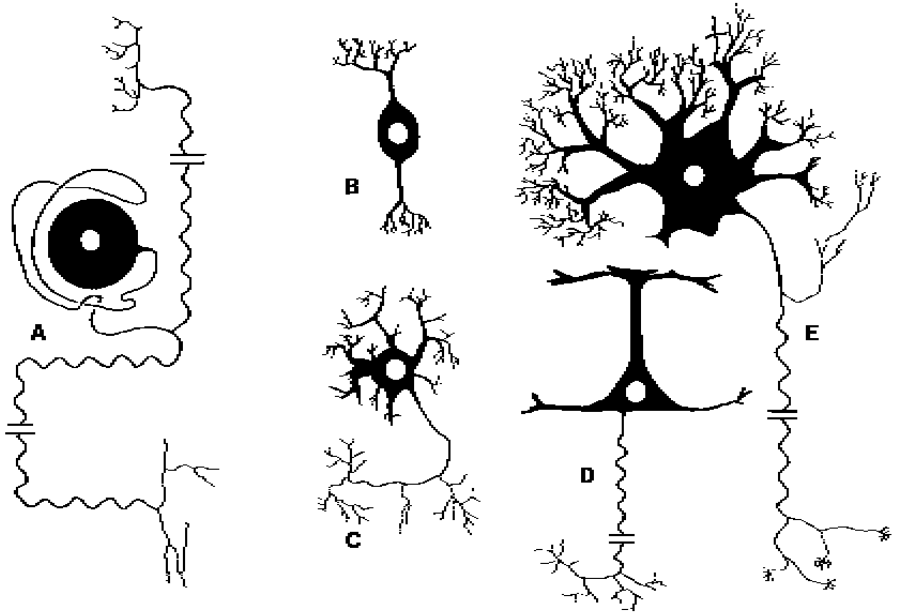


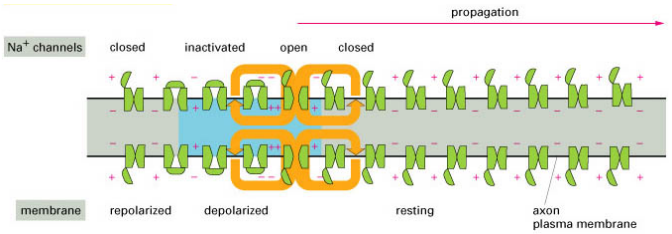
# Task 1.4: Biochemical Wires

**Motivation: Cells are not well-stirred chemical reactions**

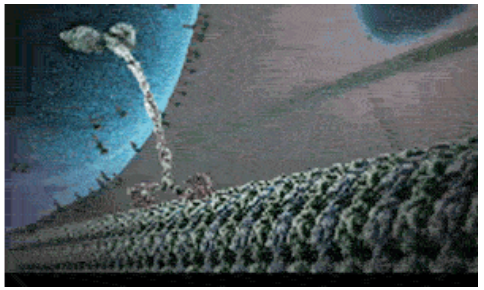
- Spatial localization of chemical reactions enables highly complex function




electrochemical waves, action potentials




physical transport



**New technical ideas - can we implement our biochemical circuits such that:**

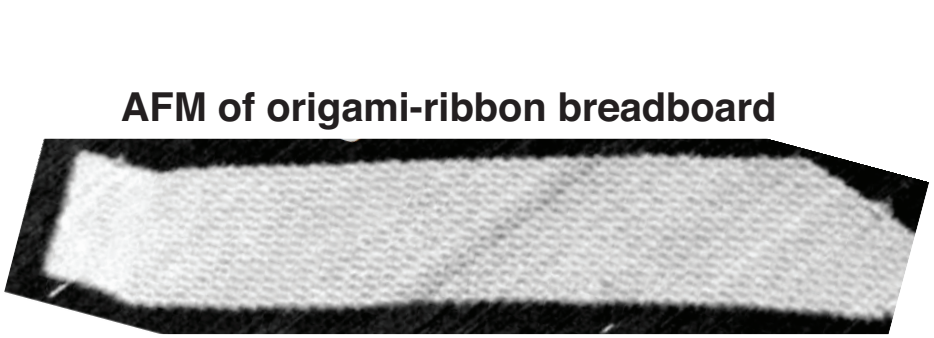
- Components are spatially isolated and can be reused in complex circuits
- Signals can propagate super-diffusively, in a directed fashion, on 1D wires
- Signals along wires can exhibit complex temporal dynamics (e.g. oscillations)
- Biochemical circuits are used to drive pattern formation

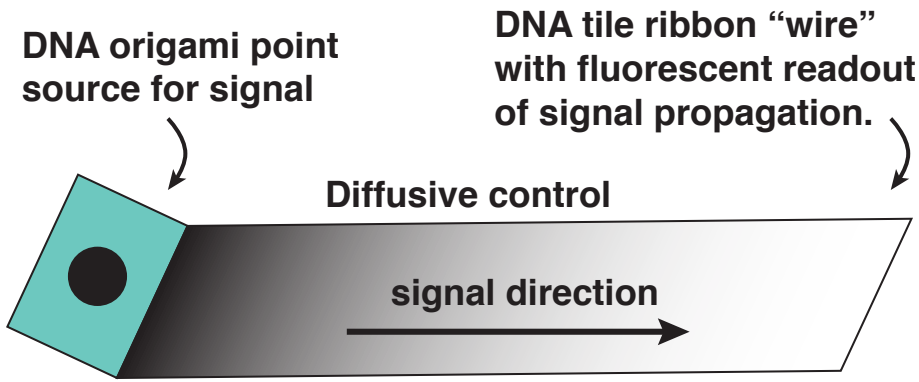
# Biochemical Wires: Phase I Objectives

**Q2: Demonstrate a transcriptional element that can be directly activated by the output of an upstream transcriptional element**

**Q4: Demonstrate signal propagation along a model biochemical wire:**

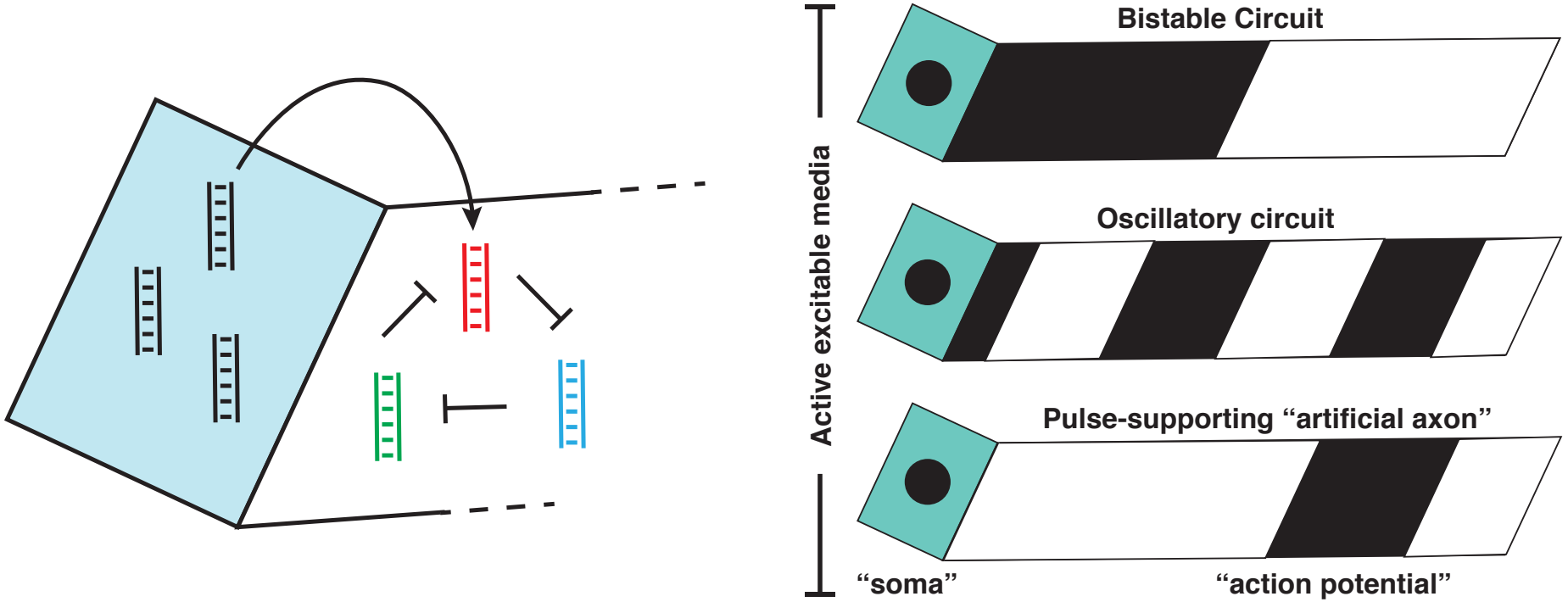
1. A DNA origami will be used as the signal source
2. A DNA tile ribbon grown from the edge of an origami will be used as a wire
3. Genelets on origami produce RNA transcripts that bind fluorescent reporters along length of ribbons (passive signals)
4. Genelets along length of ribbon are activated, and in turn, output new RNA transcripts (active signals)




Optimization of origami/ribbon growth, demonstration of fluorescence microscopy assay, quantitative comparison of passive and active signals, tuning of diffusion constants, tuning of RNA degradation/ isolation, isolation using tubes rather than ribbons...

# Biochemical Wires: Phase II Objectives



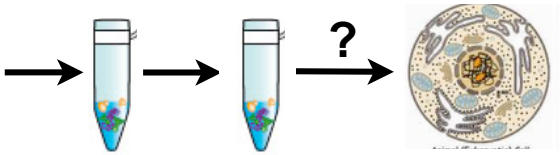

**Use RNAs produced *in vivo* to construct biochemical wires in cells**

- Demonstration of more complex circuits to add temporal dynamics
- Migration to RNA origami and RNA tile ribbons which can be synthesized *in vivo* (using RNA origami from other Living Foundries projects)
- Addition of transcription and translation, propagation of protein signals or protein modification signals (e.g. kinase cascades)

# Breadboard Project Risks and Needs

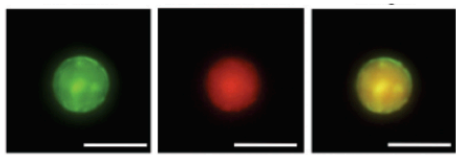
**Cell-free breadboard**

- Basic ops: low risk
- Q: are complex circuits amenable to this type of prototyping/debugging?
- Phase I: demo simple circuits (6-8 promoters)
- Phase II: demo complex circuits (8-16 promoters)




**Artificial Cells**

- Basic ops: low risk
- Q: can we implement useful mechanisms for input and output?
- Phase I: inducers, cyto-skeletal proteins
- Phase II: not currently funded



**Biochemical Wires**

- Basic ops: high risk
- Q: will TX-TL machinery work with XNA origami?
- Phase I: initial prototype of wires that localize gene products/reactions
- Phase II: attempt *in vivo* operation on RNA

**What we need from others**

- Help in trying out the protocols and identifying things that work and don't work
  - Protocols available on web: http://www.openwetware.org/wiki/breadboards
  - Workshops in Phase II, but happy to work with individuals at any time
- Larger collection of *in vitro* reporters (bulk + droplets); faster response times
- Better methods for droplet-based assays and manipulation
- RNA scaffolds/origami for trying out biochemical wires in cells

# Biomolecular Breadboards for Prototyping and Debugging Synthetic Biocircuits

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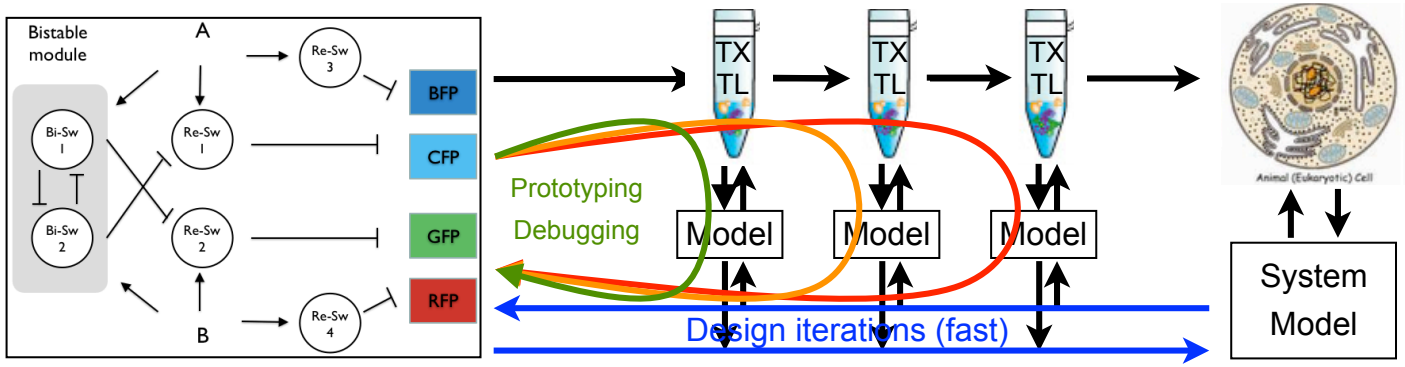


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